International Journal of Hygiene and Environmental Health 217 (2014) 515–527

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Trace metal concentrations measured in blood and urine of adolescents in Flanders, Belgium: Reference population and case studies Genk-Zuid and Menen

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ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form 4 October 2013
Accepted 4 October 2013

Keywords:
FLEHS
Trace elements
Human biomonitoring
Reference values
Genk-Zuid
Menen

ABSTRACT

In the Flemish human biomonitoring programme FLEHS II (2007–2011) trace metals (Cd, Pb, Cr, Ni, Cu, Mn, Tl, Sb, As and toxic relevant arsenic) were analysed in the blood and urine of adolescents (14–15 years old) in the reference population in Flanders and in areas of important industrial activities: Genk-Zuid and Menen. After adjustment of the results for confounding factors, the adolescents living in Genk-Zuid had higher levels of Cr, Cu and Tl in blood, higher levels of Cd and toxic relevant arsenic (TRA) in urine, but lower levels of Ni in blood and Sb in urine compared to the reference population. In Menen higher levels of Cd and Cu in urine, higher concentrations of Tl in blood but lower concentrations of Pb in blood and lower Ni, Sb and As in urine were found compared to the reference population. For both the reference population and the hot spots the concentrations are within the ranges found in other countries. Compared to the previous biomonitoring programme FLEHS I (2002–2006) a decrease in the concentrations of Cd and especially of Pb in blood was observed. However, it cannot be excluded that differences between the two campaigns are partially due to different sampling strategies.

Introduction

High concentrations of toxic metals are found in the environment, especially in areas of industrial activities. Machender et al. (2011) showed that concentrations of trace metals in the soils surrounding the industrializing area of Balanagar in Hyderabad (India) were increased by anthropogenic sources. A review article by Kabir et al. (2012) pointed out similar results: most of the areas with smelting and metal production industries (Pb, Zn, Ni, Cu, Fe and As), textile industry (Mn and Cd) and leather industry (Cr) contained significant enrichment of metal concentrations in soil. In such industrial areas, humans are heavily exposed and can accumulate high levels of trace metals (Schroijen et al., 2008; Wilhelm et al., 2007). Flanders is a region with many industries using and emitting large quantities of metals. Measurements of trace metals associated with fine atmospheric particles (PM_{10}) in the areas of Genk-Zuid and Menen show correlations between the distance from the industrial site and concentrations of trace metals attached to the PM_{10} particles (VMM, Vlaamse Milieumaatschappij, 2009). High concentrations of metals in the sediments of the Leie river at the border between France and Belgium originating from the industrial activities in Northern France have also been found (Wartel et al., 2005).

In 2002, a large-scale human biomonitoring programme (FLEHS I, 2002–2006) started in Flanders. The project was implemented by the Flemish Centre of Expertise on Environment and Health, which was funded and steered by the Flemish government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy). Within this Centre, researchers from Flemish universities (Antwerp, Brussels, Ghent, Hasselt and Leuven) and two research institutes (the Flemish Institute for Technological Research, VITO and the Provincial Institute for Hygiene, PIH) provided different sorts of expertise: medical, environmental, statistical and social

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http://dx.doi.org/10.1016/j.ijheh.2013.10.001

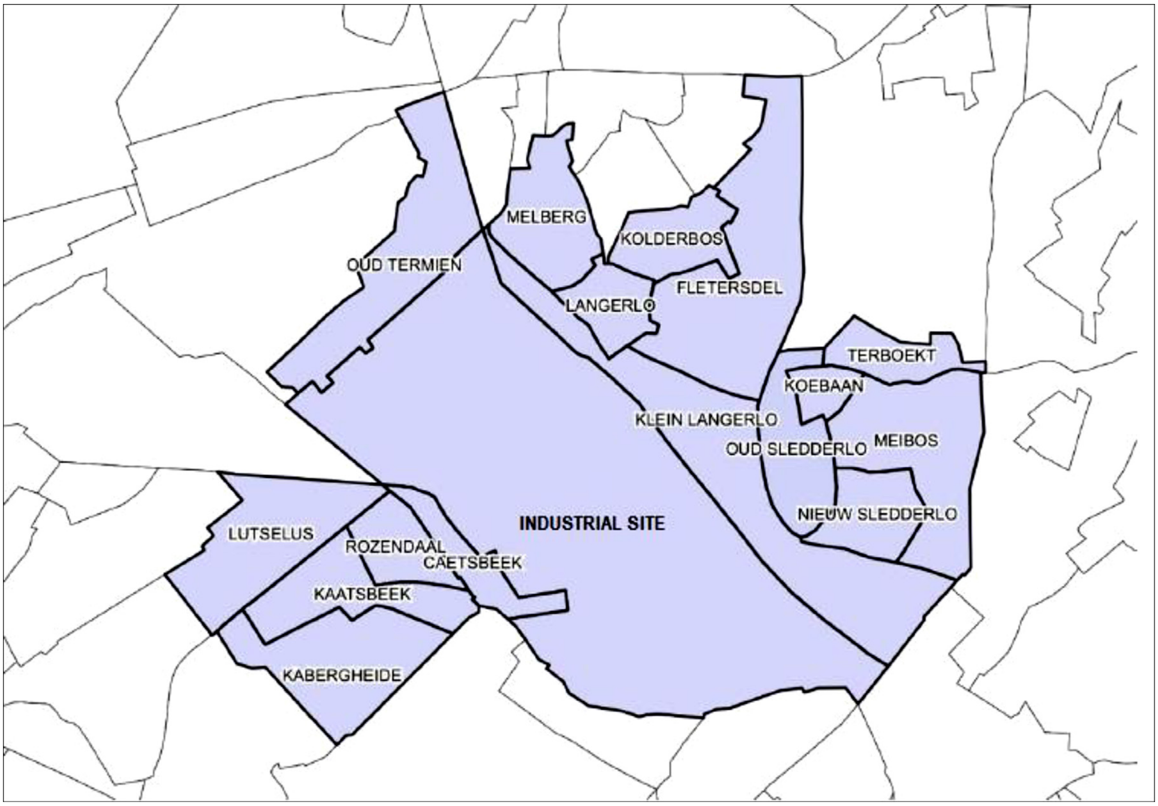

Fig. 1. Study area of Genk-Zuid.

scientific knowledge. The aims of this project were to measure internal exposure to pollutants in areas differing in pollution pressure and to assess whether place of residence or observed differences in internal concentrations of pollutants were associated with biological and health effects.

As part of the 'Decree on Preventive Health Care as a legal recognition of environmental health', voted by the Flemish government in 2003, the Flemish human biomonitoring programme was continued in 2007 with a second cycle (FLEHS II). The main purpose of this new campaign (2007–2011) was generating reference values for several biomarkers in three different population groups (adolescents, adults and mother/newborn pairs), and determining the pollution pressure in the selected hot spot areas (i.e. geographical areas or population groups with a concern for environmental pollution pressure) (Schoeters et al., 2012). The selected hot spot areas were the industrial areas of Menen and Genk-Zuid. They were selected as priority cases based on an open procedure consisting out of a multi-criteria analysis of expert assessment and stakeholder advice (Keune et al., 2010). More detailed information can be found on the website of the Flemish Centre of Expertise on Environment and Health: http://www.milieu-en-gezondheid.be.

In this paper, we will discuss the results for trace metals of this second large scale survey FLEHS II for adolescents living in the industrial areas and the general Flemish adolescent population. The correlations between metal concentrations and health effects will be discussed elsewhere.

Study design

Selection of study areas

The hot spot Genk-Zuid is a large industrial area in the South-East of Flanders with residential area in the immediate surroundings. The industrial area contains a large stainless steel plant emitting large amounts of Ni, Cr, Mn and Cd to the atmosphere. In 2007, a health survey was conducted in this region by the Provincial Institute for Hygiene. Adults aged 20–70 years, living in the area surrounding the industrial site of Genk-Zuid were interrogated (Provincial Institute for Hygiene, 2007). The health survey showed a clear difference in self-reported health conditions between the people living in the immediate surroundings of the site and those living further away from the site.

In the present study, selection of the sampling area is done by taking into account the available environmental data such as trace metal concentrations on fine atmospheric particles PM_{10} (data VMM), deposition measurements and modelling taking into account predominant wind directions. A third parameter used for the selection of the participants is the population density of the adolescents (14–15 years). The selected districts are presented in Fig. 1 and are part of the commune of Genk and the commune of Diepenbeek.

The hot spot Menen is situated in the South-West of Flanders at the border between Flanders and France, a densely populated and industrialized area. In Menen, a shredder and waste incineration oven are present. In the past, high concentrations of dioxins and PCBs were found. Exposure to metals is largely due to past and present industrial activities in Northern France. Metallurgy, chemistry, textile, paper and food industry are important industrial sectors in the region. At the banks of the Deûle river, a tributary of the Leie river, passing Menen, one of Europe's biggest Pb and Zn smelters was located. The plant, located at Noyelles-Godault (Metaleurop), had a yearly production of 170,000 tons of lead, 105,000 tons of Zn, 400 tons of Cd and 210 tons of Ag before it was closed down in 2003. This plant used a pyrometalurgic process, which generates a significant amount of dust and important atmospheric emissions of metals (DRIRE, 1998; Sterckeman et al., 2002).

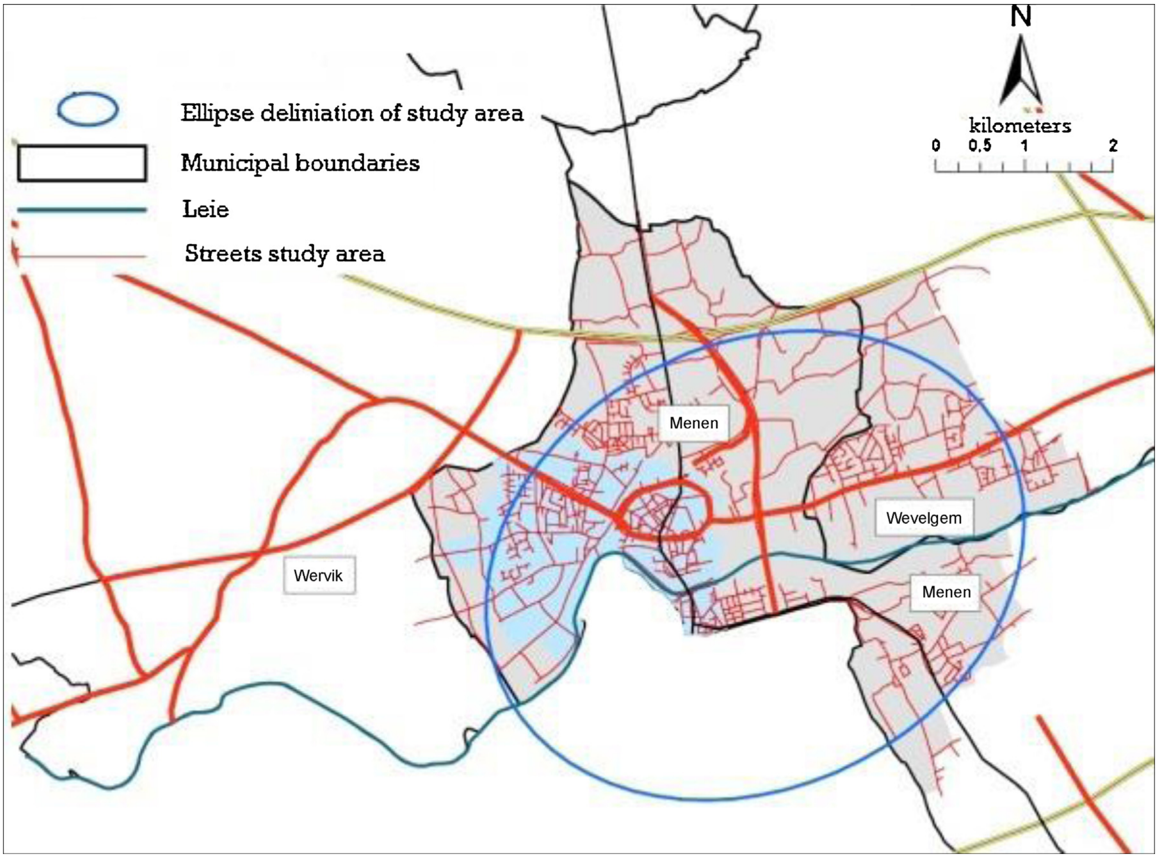

Fig. 2. Study area of Menen.

Moreover, the slags were piled in an outside heap, and carried by the wind and deposited in the surroundings. The soils around the lead and zinc smelter at Metaleurop are contaminated by metals with Cd, Pb and Zn as most abundant contaminants but also elements such as Cu, Ag and Tl (Sterckeman et al., 2000, 2002). High concentrations of metals have been observed in the sediments of the Deûle river as well as in the Leie river.

The sampling strategy is comparable to that of Genk-Zuid. First, an inventory is made of (1) all relevant industrial activities; (2) the major pollutants emitted into the environment; (3) the available environmental and human biomonitoring data, the present industry and environmental pollutants, available environmental data and results from other biomonitoring studies. Further selection is done via the population density. Fig. 2 presents the study area of Menen.

Selection of study population

The FLEHS II human biomonitoring programme focuses on establishing reference values for a wide range of biomarkers of exposure in a representative sample of the Flemish population. 14–15 year old adolescents are chosen because they may reflect recent exposure during youth (compared to adults who show a cumulative exposure for certain pollutants like PCDD/Fs and PCBs), and are thus a more interesting group for investigating the effect of the current industry in the hot spot region on the health status of people living in that area. In addition, complicating influences such as occupational exposure, commuting, changing of residence, etc. is limited in this age group. Power calculations based on the standard deviations of biomarker data from FLEHS I allowed to estimate that 200 participants per study group is sufficient to detect 30% differences between geometric means of study groups for most biomarkers that are studied ($\alpha = 0.05$, $\beta = 0.80$).

For the reference adolescent population, participants are recruited from all five Flemish provinces, proportional to the size of the population on the first of June 2007. A stratified clustered multi-stage design is used to select participants. Schools are used for adolescents ($n = 200$) as primary sampling units (PSU) for practical reasons and to maximize the participation rate. The selected PSUs are at least 20 km distant from each other. Within each PSU, individuals are randomly selected. To account for seasonal variation, recruitment is spread over one year (May 2008–July 2009). The adolescent group is stratified for sex and educational level. Inclusion criteria are: (1) residing at least 10 years in Flanders; (2) giving written informed consent, and (3) being able to fill in an extensive Dutch questionnaire. Immigrants are included in the study provided good language ability.

Because this study wants to compare exposure in Genk-Zuid and Menen with exposure in the general population of Flanders, the choice of the populations of Genk-Zuid and Menen is determined by the composition of the reference population. In the study design of Genk-Zuid and Menen, 200 adolescent samples are collected. Everybody living in the defined study area was a possible participant provided that he/she belongs to the age category (14–15 years), and is living in the selected districts for at least 5 years. As for the reference population, schools are used as primary sampling units.

All participants completed an extensive questionnaire, assessing information on lifestyle, health status, food consumption, use of tobacco and alcohol, residence history and education. The study protocol was approved by the ethical committee of the University of Antwerp.

Selection of pollutants

In FLEHS II, the following pollutants are determined: the heavy metals Cd, Pb, Ni, Cr, Mn, Cu, As, TRA, Tl, Sb, Hg and monomethylmercury (MeHg); persistent chlorinated compounds, brominated flame retardants, bisphenol A, phthalates, perfluorinated compounds, volatile organic compounds, polycyclic aromatic hydrocarbons (PAHs), organophosphate pesticides and personal care products (musks, triclosan and parabens). In this publication, only the results of the trace metals excluding Hg are discussed.

Additional parameters measured

The collection of blood and urine from the participants in this study is performed according to standard procedures. However, within these standard procedures, sample collection might be subjected to different conditions of fieldwork (e.g. time of collection, fasting conditions). Therefore, parameters of sampling are recorded and tested as covariates in the statistical analysis. Additionally, serum ferritin is determined. Ferritin is an indication of the iron status of the blood sample. This is important for the interpretation of cadmium and lead levels in blood, because a lower iron status is often correlated with a more efficient uptake of these trace elements.

Considering trace element determination in urine, ideally, 24 h urine samples would be used, but this would result in a high burden of the participants and consequently a low participation rate. As an alternative, spot urine (one time collection) samples are collected, and therefore the dilution of the urine has to be taken into account. Both creatinine and urinary density are a measure of the degree of dilution of the urine sample. Since this study is performed with adolescents aged 14–15 years, in the midst of their puberty, the body composition can vary widely in the population. Since urinary density is less dependent on the body composition, this parameter is used for the correction of the degree of dilution of the urine samples, and not creatinine.

Sample handling

Sample collection and handling

Urine and blood samples are collected via schools and, additionally in Genk-Zuid and Menen, via local district centres. Blood is sampled by a study nurse at location. The adolescents have to collect morning urine in a designated recipient, which they deliver the same day to their school.

Height and body weight of the adolescents are measured by a study nurse. Each participant donated a urine sample of about 200 mL and a blood sample of 42 mL for subsequent analysis. 10 mL of the blood sample is directly collected in a 10 mL metal free polypropylene (PP) Vacutainer with EDTA. Within 2 h after blood collection, 2 mL of whole blood is transferred from the Vacutainer into metal free PP tubes (cleaned in advance in a four steps cleaning procedure: first an ethanol–water bath (1:9, v/v; 24 h), second an acid bath (10%, v/v distilled HNO_3, 24 h), third another acid bath (10%, v/v distilled HNO_3, 24 h) and fourth a Milli Q water bath (24 h) and stored at room temperature. Within 10 h after collection, these blood samples are stored at −20 °C. The rest of the 40 mL blood sample is collected in coagulation tubes (3× 10 mL), and immediately centrifuged of the coagulated blood to obtain the necessary volume of serum (15 mL) used for different analyses (e.g. ferritin), and finally stored at −20 °C.

Morning urine is collected in a high density polyethylene container with PP cap, appropriate for trace metal analyses. During the fieldwork, 10 mL of urine is directly transferred to metal free 50 mL PP tubes, kept at 4 °C as long as the fieldwork lasted, and finally stored at −20 °C till the moment of analyses.

Analyses of trace elements in whole blood

Whole blood samples for trace metal analyses (Pb, Cd, Mn, Cu, Tl and As) are digested with HNO_3/H_2O_2 in a microwave oven using the following procedure (Schroijen et al., 2008): 500 μL blood is digested after adding 500 μL concentrated nitric acid (HNO_3; 65% distilled) and 100 μL hydrogen peroxide (H_2O_2; 30% Suprapure®, Merck, Germany) in a closed-vessel system (PFA-tubes). The samples are then subjected to a stepwise increase in temperature and pressure in a microwave system (CEM Mars5). After digestion, the samples are diluted to 5 mL and stored in acid cleaned polypropylene (PP) vessels. Analyses are performed using a high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS ELEMENT2, Thermo Finnigan, Bremen, Germany). Cr and Ni analyses in whole blood are performed after an acid digestion. An incubator at 80 °C is used to digest 500 μL of whole blood with addition of nitric acid. Analyses are performed with a Dynamic Reaction Cell DRC-ICP-MS (Perkin Elmer). Standard addition calibration is used to quantify the metals in blood.

Analyses of trace elements in urine

Metals in urine (Cd, Cu, Tl, Cr, Ni en Sb) are determined using a modification of the method described by Heitland and Köster (2004). 100 μL distilled nitric acid (ultrapure 65%, pro analyse, Merck) is added to 500 μL of urine to break down the organic matrix. Then the sample is diluted to 5 mL by adding Milli Q water. The analyses are performed using a HR-ICP-MS. Arsenic in urine is measured by Dynamic Reaction Cell (DRC)-ICP-MS (Perkin Elmer). Toxic relevant arsenic consisting of As (III), As (V), monomethylarsenic (MMA) and dimethylarsenic (DMA) is measured in urine with flow injection – hydride generation – atomic fluorescent spectrometry (PSA Analytical). The relevant toxic arsenic fraction (TRA) is in fact a good estimate of the sum of As (III), As (V), MMA, and DMA in sea fish and sea fish is a major exposure route of TRA (De Gieter et al., 2002; De Gieter and Baeyens, 2005). Therefore, we decided to include TRA in the human biomonitoring programme of Flanders.

The quality control of the TRA analyses by hydride generation-atomic fluorescence spectrometry consisted of following actions: (1) control of flowcharts of blanks included in each batch of samples; (2) control of flowcharts of the Clinchek® Urine Control Certified Reference Material included in each batch of samples; (3) participation in the G-EQUAS programme (The German External Quality Assessment Scheme for Analyses in Biological Materials).

Standard addition calibration is used to quantify the metals in urine.

Statistical analysis

Database management and statistical analyses are carried out using SAS for Windows (version 9.1.3). The trace metals are subjected to natural Neperian logarithmic transformation. Geometrical mean (GM) urinary and blood levels of Genk-Zuid/Menen and the reference population are reported and compared by means of analysis of variance (ANOVA). Differences in 90th percentiles (P90) are determined via quantile regression. Furthermore, concentrations in the adolescent populations are compared with international health guidelines by expressing the percentages of participants above these limits. The significance of differences in these percentages between Genk-Zuid/Menen and the reference population are determined via chi-square tests.

Table 1
Confounding factors and covariates for trace metals in blood and urine.

	Matrix	Genk-Zuid	Menen
Cd	Blood Urine	Age, gender, smoking, education type and serum ferritin Age, gender, smoking, density and duration urine collection	Age, gender, smoking, education type and serum ferritin Age, gender, smoking and urinary density
Pb	Blood	Age, gender, smoking, education type, season	Age, gender, smoking, traffic exposure
Cr	Blood Urine	Age, gender, smoking *Not available in reference population*	*Not analysed in Menen* *Not available in reference population*
Ni	Blood Urine	Age, gender, smoking Age, gender, smoking, density and duration urine collection	*Not analysed in Menen* Age, gender, smoking, density and duration urine collection
As	Blood Urine	*Only measured in reference population* Age, gender, smoking, recent fish consumption, density and duration urine collection	*Only measured in reference population* Age, gender, smoking, recent fish consumption and urinary density
TRA	Urine	Age, gender, smoking, density, duration urine collection and season	Age, gender, smoking, recent fish consumption and urinary density
Mn	Blood	Age, gender, smoking and season	Age, gender, smoking
Cu	Blood Urine	Age, gender, smoking, education type and season Age, gender, smoking, density and duration urine collection	Age, gender, smoking, education type Age, gender, smoking, urinary density
Tl	Blood Urine	Age, gender, smoking, highest education in family and season Age, gender, smoking, density, duration urine collection and season	Age, gender, smoking Age, gender, smoking, density and duration urine collection
Sb	Urine	Age, gender, smoking, density and duration urine collection	Age, gender, smoking, education type, highest education in family, density and duration urine collection

Multiple linear regression models are built to investigate differences in the effect of the area of living on geometrical mean trace metal concentrations between the hot spots regions Genk-Zuid/Menen and the reference population. Raw data are adjusted by regression analyses for some pre-specified, literature-based confounders (Ukkola et al., 2001; Vermeulen et al., 1996; Zitzmann and Nieschlag, 2001) and for covariates which are reported to have an influence on trace metal levels in blood and/or urine. Via a stepwise procedure, pre-specified confounders (age, gender, smoking and urinary density) and covariates (e.g. education type, fish consumption) were brought into the model. Confounders were kept in the models, non-regardless their significance. Covariates, however, were only kept in the model when their effect on the trace metal concentration was significant at the 0.10 significance level in single regression analyses. Table 1 gives an overview of the different metals and their confounding factors for blood and urine that are taken into account.

Finally, environmental data (delivered by VMM) are used to evaluate the internal concentrations of trace metals. Therefore, distances and angles to measurement stations of the VMM are calculated for each participant of Genk-Zuid and Menen. Linear regression models are used to examine whether geometrical mean blood and urine concentrations are significantly correlated with the distance from and/or angle to the measurement stations. Furthermore, immission data from the same measurement stations, determined in the period preceding sampling of blood and urine, are correlated with internal exposure marker concentrations via linear regression. For each sampling day, an average immission level is calculated based on the three day prior to the day of sampling. Each participant gets assigned an immission value, and a correlation is determined between the immission values and the urinary and blood trace metal concentrations.

Statistical significance is based on a 5% level of significance.

Quality control

Table 2 gives the detection limits (LOD), quantification limits (LOQ), average blank and standard deviation (SD) of the blank of the analysed elements in blood and urine. In every digestion cycle, two blanks are prepared and handled in the same way as the real samples, and are therefore called "procedural blanks". These procedural blanks are calculated for each batch, and distracted from the samples measured in that batch. Based on the interquartile range (IQR), extreme blanks are excluded: blanks falling out of the interval in-between lower and upper warning limits are considered as extreme values and are not taken into account.

$$\text{Lower warning limit} = 25\text{th percentile} - 1.5 \times \text{IQR}$$

$$\text{Upper warning limit} = 75\text{th percentile} + 1.5 \times \text{IQR}$$

The LOD and LOQ are calculated by taking respectively three and ten times the standard deviation of the procedural blank. This result is multiplied with the dilution factor to express the detection limit in μg/L blood and μg/L urine. The LODs of Pb, Cd, Cr, Ni, Mn, Cu, Tl and As in blood are respectively 1.9 μg/L, 0.064 μg/L, 0.25 μg/L, 0.083 μg/L, 0.64 μg/L, 1.9 μg/L, 0.002 μg/L and 0.028 μg/L. The LODs of Cd, Cu, Tl, Cr, Ni, Sb, As and TRA in urine are respectively 0.019 μg/L, 0.405 μg/L, 0.005 μg/L, 0.067 μg/L, 0.191 μg/L, 0.040 μg/L, 1 μg/L and 0.3 μg/L.

As a quality control, the method is validated by analysing certified reference materials (CRMs) in every batch of samples (Seronorm™ Trace Elements Whole Blood level 1 and 2, and, Sero A.S., P.O. Box 24, N-1375 Billingstad, Norway). In urine, only one level of Seronorm™ is available (Seronorm™ Trace Elements Urine). Additionally, the Clinchek® Urine Control CRM is used for urinary As and TRA method validation by AML, but these results were not obtained. External quality control is successfully applied by participating in the G-EQUAS interlaboratory tests in the environmental concentration range (urine: Cd, Cr, Ni and As; blood: Cd and Pb) and in the occupational exposure range (urine: Cd, As, Cu, Tl and Sb; blood: Cd, Pb and Mn). Recoveries of the CRMs are presented in Table 2. Further information on the validation of the methods can be found in the article by Vrijens et al. (2011).

In the reference population, chromium was analysed in both blood and urine. These analyses were added to the study protocol in a later stadium than the other elements because of the interest for chromium associated with the hot spot Genk-Zuid. Therefore, supplementary samples from the reference population, that were stored in our biobank, had to be used. However, these urinary

Table 2
LOD, LOQ and quality control data of blood and urine analyses.

Element	Matrix	LOD (μg/L)	LOQ (μg/L)	Average blank (μg/L)	SD blank (μg/L)	CRM1 recovery (%)	CRM2 recovery (%)
Cd	Blood	0.064	0.21	0.003	0.002	99	105
	Urine	0.019	0.064	0.002	0.001	90	–
Pb	Blood	1.9	6.3	0.13	0.063	111	108
Cr	[a]Blood	0.25	0.85	0.038	0.004	–	–
	Urine	0.067	0.22	0.017	0.004	94	–
Ni	[a]Blood	0.083	0.28	0.023	0.001	–	–
	Urine	0.19	0.64	0.042	0.020	97	–
As	Blood	0.028	0.093	0.002	0.001	99	86
	[a]Urine	0.074	0.25	0.20	0.002	–	–
TRA	[a]Urine	0.68	2.3	0.13	0.005	–	–
Mn	Blood	0.64	2.1	0.054	0.021	107	91
Cu	Blood	1.9	6.4	0.18	0.064	97	94
	Urine	0.40	1.3	0.095	0.030	90	–
Tl	Blood	0.002	0.007	0.0001	0.00007	–	125
	Urine	0.005	0.017	0.0004	0.0003	112	–
Sb	Urine	0.040	0.13	0.005	0.002	99	–

[a] Analysed by Algemeen Medisch Labo (AML), Antwerp.

biobank samples showed abnormally high chromium levels, which forced us to discard those results.

Results

Overview study population

To obtain a good statistical power, the number of participants has to be approximately the same for the three populations. The number of participants in the reference population, Genk-Zuid and Menen were, respectively 210, 197 and 199. The respective average age was 14.8 ± 0.5, 15.0 ± 0.7 and 15.1 ± 0.8 years.

Sampling conditions

A comparison of the influencing sampling conditions in the reference population, Genk-Zuid and Menen is given in Table 3. Significantly higher serum ferritin concentrations are found in Menen ($p < 0.001$), whereas for Genk-Zuid, no significant difference with the reference population is observed. Urinary creatinine is significantly higher ($p = 0.004$) in Genk-Zuid compared to the Flemish reference population. The urinary density, however, is not significantly different between these two populations. In Menen, the average urinary creatinine was also significantly increased compared to adolescents from Flanders ($p = 0.003$).

Trace elements in blood and urine: unadjusted (raw) data

Unadjusted trace metals concentrations in blood and urine of the three populations are presented in Table 4. For almost all trace metals, the concentrations in blood and urine are above the LOD. The lowest percentage of samples with a value above the LOD, i.e. 60%, is observed for urinary antimony (U-Sb) in the Menen population.

Geometrical mean blood cadmium (B-Cd) levels are significantly higher in Genk-Zuid compared to the reference population. In the population of Menen, no significant difference is found for B-Cd in comparison to the reference population.

In Genk-Zuid, urinary cadmium (U-Cd) values are significantly higher than in the reference population. The 90th percentiles of the reference population and Genk-Zuid are respectively 0.51 and 0.61 μg/L; these differences are borderline significant. U-Cd values in Menen are significantly higher than in the reference population. The 90th percentiles are respectively 0.61 μg/L and 0.51 μg/L, but the difference is not significant.

The mean blood lead concentration (B-Pb) in Genk-Zuid is lower than in the general adolescent population of Flanders, but this difference is not significant. The population of Menen has a geometrical mean B-Pb concentration of 12.9 μg/L, which is significantly lower than the reference population (14.8 μg/L). The P90 values for B-Pb are not significantly different.

In Genk-Zuid, chromium is measured in both blood and urine, in Menen only in urine. The geometrical mean chromium concentration in blood (B-Cr) is significantly higher in Genk-Zuid (0.33 μg/L) than in Flanders (0.26 μg/L). The 90th percentiles do not differ significantly between the two populations.

Nickel is determined in blood and urine of the reference population and in Genk-Zuid. In Menen, only urinary nickel is determined. When comparing blood nickel (B-Ni), a significantly lower value is observed for Genk-Zuid in relation to Flanders: the geometrical means are 1.15 μg/L and 1.25 μg/L respectively. The 90th percentile does not differ significantly between the two regions. For the urinary nickel (U-Ni) concentrations of Genk-Zuid (2.42 μg/L), no significant difference is found with concentrations in the reference population (2.58 μg/L). Comparing U-Ni of Menen (2.01 μg/L) with this reference value, a significantly lower geometrical mean can be found in Menen.

Blood arsenic (B-As) is only measured in the adolescents of the reference population. In Genk-Zuid and Menen, urinary total arsenic (U-As) and urinary TRA (U-TRA) are used as markers of arsenic exposure. Concentrations of U-As are not significantly different between Genk-Zuid (12.2 μg/L) and the reference population (12.1 μg/L). For TRA however, concentrations in urine (U-TRA) are significantly higher in Genk-Zuid (6.5 μg/L) than in Flanders (4.7 μg/L). In Menen, neither U-As (11.2 μg/L) nor U-TRA (4.8 μg/L) is significantly different compared to the reference population.

Manganese is analysed in blood (B-Mn) in the three different adolescent populations. The reference value (9.7 μg/L) is not significantly different from the values of Genk-Zuid (10.0 μg/L) and Menen (9.9 μg/L).

Copper is determined in both blood and urine. Blood copper concentrations (B-Cu) in Genk-Zuid (839 μg/L) and Menen (835 μg/L) are significantly higher than in the reference population (790 μg/L). Also urinary geometrical mean copper concentrations

Table 3
Sampling conditions.

Parameters	Reference population	Genk-Zuid	Genk-Zuid vs. ref.	Menen	Menen vs. ref.
Blood samples					
Serum ferritin (μg/L)					
Average (SD)	32.6 (16.9)	29.8 (20.8)	$p = 0.14$	43.8 (27.9)	$p < 0.001$
Median (min.–max.)	29 (2–100)	26 (2–189)		38 (4–187)	
Urine samples					
Duration coll. (min)					
Average (SD)	522 (161)	487 (203)	$p = 0.06$	513 (232)	$p = 0.67$
Median (min.–max.)	550 (35–1380)	540 (30–1197)	$p < 0.001$	540 (0–1365)	$p = 0.004$
<465 min	17.4%	36.5%		24.9%	
465–545 min	30.1%	18.3%		32.6%	
465–600 min	30.6%	16.8%		15.5%	
≥600 min	21.9%	28.4%		26.9%	
Creatinine (mg/dl)					
Geom. mean (95% CI)	131 (123–139)	151 (140–163)	$p = 0.004$		$p = 0.003$
Average (SD)	143.5 (60.0)			162.5 (69.4)	
Median (min.–max.)	133.5 (20–308)			159 (24–423)	
Density (g/cm^3)					
Geom. mean (95% CI)	1.0219 (1.0210–1.0227)	1.0224 (1.0215–1.0234)	$p = 0.38$		
Average (SD)	1.022 (0.0006)			1.022 (0.007)	$p = 0.74$

(U-Cu) in Genk-Zuid (12.0 μg/L) and Menen (11.2 μg/L) are significantly higher than in the reference population (10.0 μg/L).

Thallium is measured in both blood (B-Tl) and urine (U-Tl). B-Tl was higher in both Genk-Zuid (0.029 μg/L) and in Menen (0.035 μg/L) compared to the reference population (0.027 μg/L) at the $p < 0.05$ level. Also the 90th percentile of the Genk-Zuid and Menen population are significantly higher than the 90th percentile of the Flemish control group. Urinary Tl in the hot spot areas shows a similar pattern as B-Tl: significantly higher concentrations in Genk-Zuid (0.24 μg/L) and Menen (0.25 μg/L) compared to Flanders (0.20 μg/L). Again, the 90th percentiles are significantly higher in Genk-Zuid and Menen.

Table 4
Unadjusted[a] trace metals: geometrical means (GM) and 90th percentiles (P90) of trace metals in blood (B) and urine (U) in the hot spots Genk-Zuid and Menen, and the reference population.

		Unit (μg/L)	Reference pop.	Genk-Zuid	p	Menen	p
Cd	B	GM (95% CI)	0.21 (0.19–0.23)	**0.24 (0.22–0.25)**↑	0.03	0.19 (0.18–0.21)	0.33
		P90	0.41	0.43	0.78	0.34	0.55
	U	GM (95% CI)	0.24 (0.22–0.27)	**0.30 (0.28–0.33)**↑	0.001	**0.33 (0.31–0.35)**↑	<0.001
		P90	0.51	**0.61**↑	0.04	0.61	0.06
Pb	B	GM (95% CI)	14.8 (13.9–15.7)	13.7 (12.9–14.5)	0.08	**12.9 (12.3–13.6)**↓	<0.001
		P90	25.1	23.8	0.63	21.7	0.09
Cr	B	GM (95% CI)	0.26 (0.24–0.28)	**0.33 (0.31–0.36)**↑	<0.001	–	–
		P90	0.50	0.59	0.08	–	–
	U	GM (95% CI)	–	0.27 (0.25–0.29)	–	0.27 (0.24–0.29)	–
		P90	–	0.53	–	0.56	–
Ni	B	GM (95% CI)	1.25 (1.19–1.31)	**1.15 (1.11–1.20)**↓	0.02	–	–
		P90	1.66	1.64	0.88	–	–
	U	GM (95% CI)	2.58 (2.29–2.91)	2.42 (2.20–2.65)	0.39	**2.01 (1.82–2.22)**↓	0.002
		P90	5.70	5.30	0.56	4.88	0.34
As	U	GM (95% CI)	12.1 (10.6–13.9)	12.2 (10.7–13.9)	0.79	11.2 (9.6–13.2)	0.58
		P90	43.9	48.5	0.74	46.7	0.87
TRA	U	GM (95% CI)	4.66 (4.09–5.31)	**6.47 (6.01–6.97)**↑	<0.001	4.8 (4.3–5.3)	0.77
		P90	10.3	11.4	0.24	9.4	0.41
Mn	B	GM (95% CI)	9.66 (9.28–10.1)	9.98 (9.59–10.40)	0.25	9.94 (9.57–10.33)	0.30
		P90	13.8	14.1	0.65	13.8	0.99
Cu	B	GM (95% CI)	790 (774–807)	**839 (819–960)**↑	<0.001	**835 (819–851)**↑	<0.001
		P90	913	**1010**↑	0.008	**995**↑	0.002
	U	GM (95% CI)	10.0 (9.2–10.9)	**12.0 (11.2–12.8)**↑	0.001	**11.2 (10.4–12.0)**↑	0.04
		P90	19.0	20.9	0.30	18.9	0.89
Tl	B	GM (95% CI)	0.027 (0.026–0.028)	**0.029 (0.028–0.030)**↑	<0.001	**0.035 (0.033–0.036)**↑	<0.001
		P90	0.034	**0.038**↑	0.002	**0.048**↑	<0.001
	U	GM (95% CI)	0.20 (0.18–0.21)	**0.24 (0.22–0.26)**↑	<0.001	**0.25 (0.23–0.27)**↑	<0.001
		P90	0.35	**0.43**↑	0.03	**0.45**↑	0.04
Sb	U	GM (95% CI)	0.087 (0.080–0.100)	**0.074 (0.066–0.083)**↓	0.02	**0.067 (0.059–0.075)**↓	<0.001
		P90	0.19	0.18	0.89	0.18	0.81

Significant differences are highlighted in bold (as compared to the reference population).

[a] 'Unadjusted' means that the data were not corrected for confounders such as age, gender, smoking, education type and serum ferritin.

Table 5
Comparison of trace metals levels between Genk-Zuid/Menen and the reference population after correction for confounders and covariates.

	Effect of area: Genk-Zuid vs. Flanders	p	Effect of area: Menen vs. Flanders	p
B-Cd	+10%	0.14	-3%	0.69
U-Cd	+18%	0.008	+28%	<0.001
B-Pb	-2%	0.63	-11%	0.005
B-Cr	+32%	<0.001	/	/
B-Ni	-7%	0.03	/	/
U-Ni	-8%	0.30	-25%	<0.001
U-As	+6%	0.55	-20%	0.03
U-TRA	+32%	0.001	-7%	0.40
B-Mn	+2%	0.42	+4%	0.20
B-Cu	+5%	0.009	+6%	<0.001
U-Cu	+11%	0.03	+6%	0.22
B-Tl	+11%	<0.001	+27%	<0.001
U-Tl	+8%	0.16	+14%	0.005
U-Sb	-21%	0.003	-25%	<0.001

The figures indicate how many percent the concentration in the respective study area (Genk-Zuid or Menen) is above (+) or below (−) the concentration measured in the reference population, after correction for confounders and covariates. Significant increase compared to the reference population is indicated in red, significant decrease in green.

Concentrations of antimony in Genk-Zuid and Menen are only determined in urine (U-Sb). Compared to the reference group (0.087 μg/L), these values are significantly lower in both Genk-Zuid (0.074 μg/L) and Menen (0.067 μg/L). The 90th percentiles do not differ significantly.

Comparison of trace metal concentrations with the reference values after correction for confounders and covariates

The results described above represent unadjusted (raw) results. This means that the effects of methodological factors or population characteristics on the concentrations of those metals were not taken into account. There are, however, several factors that can strongly influence these concentrations. For adolescents, the most important influencing factors are age, gender and smoking, but several others can be involved, like education type, season, duration of urine collection, serum ferritin and urinary density. In order to obtain the most reliable estimate of the blood and urine concentration without the bias from a non-desired factor, the influence of some of these parameters was statistically determined and taken into account. In some cases, correction for confounders and covariates changed the significance of the difference between Flanders and Genk-Zuid/Menen. Unadjusted B-Cd and U-Cd were significantly higher in Genk-Zuid compared to the reference population. After correction for age, gender, smoking, education type and serum ferritin, B-Cd was still 10% higher in Genk-Zuid than in Flanders, but this difference was no longer statistically significant ($p = 0.14$). An important confounding factor of U-As was recent (past 3 days) fish consumption. After correction for fish consumption and other influencing factors such as age, gender, smoking and density of the urine, the total arsenic concentration became significantly lower in the population of Menen compared to the reference population (20% lower, $p = 0.03$). Although the geometrical mean U-Cu in Menen was still 6% higher than in Flanders after adjustment for age, gender, smoking habits and urinary density, this difference was no longer significant ($p = 0.22$). Urinary Tl in Genk-Zuid was 8% higher than in the reference population after adjustment (for age, gender, smoking habits, density and duration of urine collection and season), but this result was no longer statistically significant ($p = 0.16$).

An overview of the differences between trace metal levels in Genk-Zuid and Menen compared to the Flemish reference population after correction for confounders and covariates is given in Table 5.

After correction for confounders and covariates (Table 1), living in Genk-Zuid was associated with a significantly higher exposure to cadmium (18% higher in urine), chromium (32% higher in blood), copper (5% higher in blood, 11% higher in urine), thallium (11% higher in blood) and arsenic (toxic relevant arsenic 32% higher in urine) compared to the reference population. On the other hand, significantly lower concentrations in the Genk-Zuid population were observed for antimony (21% lower in urine) and nickel (7% lower in urine). After correction for confounding factors and covariates, we could conclude that living in the area of Menen was associated with significantly higher exposure to thallium (27% higher in blood and 14% higher in urine), copper (6% higher in blood) and cadmium (28% higher in urine) in the adolescent population. Significantly lower values were observed for lead (11% lower in blood), nickel (25% lower in urine), antimony (25% lower in urine) and arsenic (20% lower in urine).

Comparison of trace metal concentrations with health guidelines

Comparison of the measured concentrations with health guidelines for non-occupational exposure to trace metals is shown in Table 6. Human biomonitoring values (HBM I and HBM II) were determined by the German Human Biomonitoring Commission (HBC) for Cd and Tl in urine (Schulz et al., 2011). The HBM I value represents the concentration above which possible health effects cannot be excluded, while HBM II values indicate the level above which an extra health risk is possible. Guidelines determined in the past for blood lead (HBM I: 100 μg/L for children) are now suspended due to findings from epidemiological studies showing health effects below 100 μg/L (Wilhelm et al., 2010). Based on recent research indicating that lead is associated with neurobehavioral damage at blood levels of 50 μg/L and even lower, also the World Health Organization (WHO) states that there appears to be no threshold level below which lead causes no injury to the developing human brain (WHO, 2010). Biomonitoring Equivalents (BE), i.e. the concentration of a chemical or its metabolite in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guideline, were determined by Hays et al. (2008, 2010) for Cd in urine and blood, and TRA in urine. For all other measured trace elements, no health guidelines were available. Comparing U-Cd in Genk-Zuid with the human biomonitoring guideline (HBM I) of 0.5 μg/L, it was observed that 20% of the measured values of Genk-Zuid exceeded this limit, while in Flanders this was 11%. Thus, background exposure concentrations of

Table 6
Comparison of measured trace metal concentrations with health guidelines (% of Genk-Zuid, Menen and the reference population above the health guidelines).

Element	Health guideline	Genk-Zuid	Menen	Ref. pop.
Urine				
Cd	0.5 μg/L (HBM I, HBC)	19.6%	19.9%	11.4%
	2 μg/L (HBM II, HBC)	0%	0%	0%
	1.2 μg/L (BE, ATSDR)	0%	0.5%	0%
	1.5 μg/L (BE, EPA)	0%	0.5%	0%
TRA	6.4 μg/L (BE, Hays et al., 2010)	64.5%	–	33.8%
Tl	5 μg/L (HBM I, HBC)	0%	0%	0%
Blood				
Cd	1.4 μg/L (BE, ATSDR)	0.5%	3.5%	1.9%
	1.7 μg/L (BE, EPA)	0.5%	3.0%	1.9%
Pb	100 μg/L (WHO)	0%	0%	0%

HBC, Human Biomonitoring Commission (Germany); ATSDR, Agency for Toxic Substances and Disease Registry; EPA, United States Environmental Protection Agency; WHO, World Health Organization.

cadmium led in 11% of the adolescent population to a urinary excretion whereby a health risk could not be excluded. In Genk-Zuid, this percentage was more or less double. This difference was borderline not significant ($p = 0.054$). There were only a few of the participants with B-Cd exceeding the BE health guideline of 1.7 μg/L (Genk-Zuid: 0.5%; Flanders: 1.9%). The BE guideline for U-TRA (6.4 μg/L) was close to the geometrical mean U-TRA of Genk-Zuid (6.5 μg/L). 65% of the participants in Genk-Zuid had a U-TRA concentration above this guideline, while in the reference population this was 34%. The guideline for U-Tl was determined by the HBC at 5 μg/L (Schulz et al., 2011). None of the adolescents of Genk-Zuid or the reference population had a U-Tl level exceeding this HBM I guideline.

Comparing U-Cd concentrations in Menen with the human biomonitoring guideline of 0.5 μg/L showed that 20% of the urinary samples were above this limit. For the reference population of Flanders, this was 11%, which was significantly lower than in Menen ($p = 0.04$). For B-Cd, 3% of the population had a value above the BE 1.7 μg/L, which was not significantly different from the reference population (1.9%). No adolescents of Menen had U-Tl concentrations above the HBM I guideline.

Correlations with environmental data

To further support the results presented above, correlations between urinary/blood levels and environmental data obtained from VMM were examined.

For each participant of Genk-Zuid, the distance from and the angle to the measurement stations of VMM, located southeast of the industrial site, were determined, and the correlation with trace metal levels was examined. On the population level, it is observed that B-Pb levels decreased significantly with increasing distance, being highest close to the industrial site. Based on the obtained regression model, blood concentrations could be calculated in function of the distance to the measurement posts. Adolescents living at a distance of 0.5, 1.0 and 2.0 km had calculated B-Pb levels of 15.1 μg/L, 14.7 μg/L and 14.0 μg/l respectively.

Urinary and blood trace metal concentrations of adolescents from Genk-Zuid were compared to the available environmental immission data of the VMM. For chromium, the average immission value varied between 4 μg/m^3 and 187 μg/m^3 of air. Although no significant correlation was observed for B-Cr, there was a significant correlation between the Cr immission during the last three days and the U-Cr level. With an increase of 20 ng/m^3 air, the U-Cr level increased with a factor 1.03. For all other trace elements, no correlation with the emission values was observed in Genk-Zuid.

Because of the observed significant elevation of U-TRA levels in Genk-Zuid, it was examined if there were elevated TRA concentrations in the air or the drinking water of Genk-Zuid. No clear correlation was found between U-TRA and immission values of arsenic in air during the last three days prior to sampling. The drinking water data were obtained from the Vlaamse Maatschappij voor Watervoorziening (VMW) during the period of sampling. Nowhere, the drinking water standard was exceeded.

Also for the adolescent population of Menen, additional research was performed to correlate the observed trace metal levels with available data from the VMM. The correlation between individual U-Cd levels and the distance from and angle to a measurement station of the VMM was examined. In this station, PM_{10} deposition measurements were performed. Both for the distance and the angle, no correlation with U-Cd levels was observed. The same test was performed for B-Cu, but no correlations were found, not for the distance and not for the angle.

Discussions

Genk-Zuid

In 2008, high annual average concentrations of Cr, Ni, Mn and Cd were measured in fine dust particles (PM_{10}) in some of the VMM measurement stations in Genk-Zuid, all highly exceeding the annual average rural background concentrations (Cr: 5 ng/m^3; Ni: 6 ng/m^3; Mn: 14 ng/m^3; Cd: 0.4 ng/m^3). Annual average Cr levels up to 300 ng/m^3 were found, Ni levels up to 111 ng/m^3 (largely exceeding the EU regulation of 20 ng/m^3), Mn levels up to 115 ng/m^3 and Cd up to 7.2 ng/m^3 (exceeding the EU regulation of 5 ng/m^3). From 2009 onwards a decreasing trend was observed, partly due to a decrease in smelting activities, but also because of measures taken in the production processes. In 2011, only concentrations of Ni were still exceeding the EU guideline. Levels up to 32 ng/m^3 were measured. High daily variability was also observed as well as regional differences between the monitoring stations in Genk-Zuid (VMM, 2009, 2012a). Despite the high atmospheric concentrations, B-Ni was significantly lower than in the adolescents of the reference population and no difference was found in U-Ni between Genk-Zuid and the reference population. A possible explanation for this is that the nickel compounds in the airborne particles are present as compounds which are rather insoluble in water and hence are not assimilated in the human body. A study conducted in 2010 by the Flemish Institute of Technological Research (VITO) showed that the nickel fraction in the dust in Sledderlo (suburban of Genk-Zuid, see Fig. 1) existed for 78% of insoluble nickel compounds (nickel oxides) (Standaert et al., 2010). Retention of particulate nickel in the respiratory tract is thus more important than uptake in blood. The health effects related to exposure to atmospheric Ni compounds can thus not be assessed with biomonitoring.

For B-Pb concentrations, no significant difference between Genk-Zuid and the reference population were observed. However, the result regarding the distance to the measurement station of VMM indicated that adolescents living closest to the industrial site of Genk-Zuid were exposed more to Pb. Since the district near the industrial site is a social district with old infrastructure, the presence of leaden service pipes was checked through a follow-up study by the VMW. They performed Pb analyses of the drinking water at 5 addresses in Nieuw-Sledderlo to investigate whether there were elevated Pb-levels due to the presence of lead pipes. But none of the results exceeded the drinking water standard.

U-Cd is a measure for medium to long-term exposure, thus reflecting the accumulated exposure from the region. The geometrical mean concentration of U-Cd in the adolescent population of Genk-Zuid was significantly increased compared to the reference population. Also the geometrical mean B-Cd was higher in Genk-Zuid, but this significant difference disappeared after correction for confounding factors and covariates. The percentage of adolescents with U-Cd levels exceeding the HBM I health guideline was also clearly higher than in the reference population. These results are in accordance with the high emissions of cadmium associated with PM_{10} in Genk-Zuid.

Chromium, copper and thallium are markers of recent exposure. Significantly higher B-Cr levels were found in Genk-Zuid compared to Flanders. For U-Cr, no reference value was available. The elevated B-Cr concentrations were reflected in the high chromium levels measured in fine dust particles in Genk-Zuid. Furthermore, an association was found between chromium immission values from the last three days prior to sampling and U-Cr levels in adolescents.

Copper concentrations were significantly increased in blood and urine of adolescents living in Genk-Zuid. The average annual concentration of Cu associated with PM_{10} varied from 9 to 37 ng/m^3. These levels were comparable with urban background concentrations (VMM, 2012a). On the other hand, highest B-Cu concentrations were observed in adolescents living closest to the industrial area, suggesting that there was a source of Cu present in the area.

Thallium levels in adolescent blood and urine were significantly higher in Genk-Zuid compared to the Flemish reference population. After correction, the significant difference for U-Tl disappeared. Industrial activities such as metal recycling, production of construction materials, metallurgy, and coal combustion processes are important sources of thallium emission to the environment. However, no atmospheric monitoring data were available, and further research is needed to determine the origin of thallium, causing elevated blood and urinary levels.

TRA in urine reflects exposure in the last 1–2 days. Exposure to TRA generally happens through the air or via drinking water. Annual average arsenic concentrations on fine dust particles PM_{10} were low in Genk-Zuid (0.5 ng/m^3), comparable to urban background concentrations (0.4 ng/m^3) and well below the EU-guideline of 6 ng/m^3. No differences were observed between the different monitoring sites in Genk-Zuid (VMM, 2009). In addition, drinking water, which can be a source of TRA due to enrichment via soil, was checked for arsenic concentrations (data were obtained via the VMW), but As concentrations in drinking water in the districts that were studied were consistently low. Important food sources of TRA were grain and rice products and fruits. Investigation of food consumption patterns showed that less locally produced fruit, vegetables and meat were consumed by the adolescents in Genk-Zuid compared to the reference population.

In Genk-Zuid, atmospheric Sb concentrations were low (0.9–2.1 ng/m^3) and comparable to those found in remote and urban monitoring stations (0.8 ng/m^3 and 1.8 ng/m^3 respectively), and lower than other monitoring sites in Flanders (3.6–99 ng/m^3). This indicated that pollution sources (such as electronics industry and coal combustion) were significantly lower for Sb in Genk-Zuid (VMM, 2009), and explained the lower Sb levels observed in the adolescents from Genk-Zuid.

Menen

Monitoring of atmospheric pollutants in Menen is limited to organic pollutants as these are the priority pollutants in that region. There are, however, PM_{10} measurements conducted by VMM. Since trace metals can be absorbed onto these PM_{10} particles, we examined whether there exists a significant relation between U-Cd and B-Cu values of the participants and the distance and angle to the VMM measurement spot. However, no significant relations with U-Cd and B-Cu are found. Thallium is not measured by the VMM, so this relation could not be examined.

The source of metals in Menen is predominantly originating from the industrial activities in Northern France (agglomerations of Roubaix and Lille). Metals are analysed in the trans boundary rivers Leie and Scheldt in the framework of the European INTERREG-Stardust project (2000–2006). Extremely high concentrations of metals are found in the sediments of the Deûle river at the Metaleurop site decreasing slowly downstream Table 7. In the Leie river in Menen concentrations of metals (e.g. Cd, Tl and Cu) are still significantly elevated compared to the Scheldt river (Bassin Rond). Concentrations are enhanced both in the sediments and in the dissolved phase of the water column (Wartel et al., 2005). In the past, dredged sludge was deposited on the river banks and even used in agricultural soils. During flooding events, contaminated sediments are deposited in the surrounding soils. In addition, in dry periods, Leie water is sometimes used for irrigating lands. No information however is available on metal concentrations in soils in the region of Menen, nor in the locally produced vegetables. Thus, the elevated levels of U-Cd, B-Cu, U-Tl and B-Tl might be partially due to the metallurgical industry located in the North of France. However, the elevated Pb, Ni and Sb levels in the Leie river in Menen are not in accordance with the significantly lower concentrations of these elements observed in the adolescents (Table 7).

Comparison with FLEHS I and literature

Comparison of the data of the present study with previous monitoring data in Flanders (FLEHS I) and literature values are summarized in Table 8. Only Cd and Pb concentrations in whole blood are measured in both FLEHS I and FLEHS II programmes. When comparing the results of FLEHS I and FLEHS II, it is important to consider the differences in sampling strategies between both campaigns. Not only is there a dissimilarity in population size, with a greater number of adolescents in FLEHS I, there is also only a small overlap in recruited areas. In FLEHS I, the adolescents are partially coming from specific areas like harbours and regions known for their historical pollution with heavy metals, while in FLEHS II, adolescents are more homogenously distributed over Flanders. This reflects in the Cd and Pb P90 values of FLEHS II being lower and closer to the geometrical mean than the P90 values of FLEHS I, indicating that there are possible more abnormal high concentrations in the areas of FLEHS I. The reported lead value in the FLEHS II (14.8 (14.0–15.6) μg/L) is lower compared to the one observed in the first study (21.7 (20.8–22.6) μg/L). There is no overlap in the 95% CI of the geometrical mean values for both campaigns. A possible explanation could be the declining trend which has been reported during the last few decades in Europe (Smolders et al., 2010). A clear declining trend in atmospheric Pb concentrations is observed in Flanders, both in industrial and rural areas. During the last decade (2000–2011), lead emissions to air decreased significantly from 62 tons in 2000 to 26 tons in 2011. The sampling of FLEHS I took place between October 2003 and July 2004.

Table 7
Trace metal concentrations in sediments and water (dissolved phase) of Rivers Deûle, Leie and Scheldt.

Location	River Sediment	Cd (µg/g)	Sb (µg/g)	Tl (µg/g)	Pb (µg/g)	Cr (µg/g)	Ni (µg/g)	Cu (µg/g)
Metaleurop	Deûle	1400	259	227	1180			
Haubourdin	Deûle	148	4.93	89.3	603	60.3	25.3	106
Deûlemont	Deûle	20.4	5.09	88.8	230	99.5	26.7	149
Warneton	Leie	15.8	0.50	24.2	215	62.3	31.9	87.4
Menen	Leie	3.77	4.05	6.58	94.4	54.4	21.6	42.6
Bassin Rond	Schelde	1.95	1.95	0.23	47.8	76.0	11.7	16.2
Location	**River Water dissolved**	**Cd (µg/L)**	**Sb (µg/L)**	**Tl (µg/L)**	**Pb (µg/L)**	**Cr (µg/L)‘**	**Ni (µg/L)**	**Cu (µg/L)**
Haubourdin	Deûle	0.19	4.00	8.41	10.8	0.2	4.99	1.42
Deûlemont	Deûle	0.03	3.68	3.85	2.55	0.22	9.60	1.47
Warneton	Leie	0.04	3.03	2.58	2.39	0.24	10.4	1.23
Menen	Leie	0.06	3.77	2.03	3.22	0.27	10.7	1.66
Bassin Rond	Scheldt	0.03	0.24	0.01	0.78	0.29	1.94	0.78

Deûle: river in northern France, flowing into the Leie river at Deûlemont. Sampling spots were located between the Metaleurop site and Deûlemont.
Leie: left tributary of the Scheldt, with its source in Pas-de-Calais, France, flowing into the river Scheldt in Ghent, Belgium. Sampling spots were located in Warneton and Menen.
Scheldt: river in northern France, western Belgium and southwestern part of the Netherlands. Sampling spot was located in the upper Scheldt (Bassin Rond, France).

Lead emissions to air in 2003 and 2004 were respectively 43 and 54 tons. During the sampling period of FLEHS II (May 2008–July 2009), lead emissions were significantly lower with respectively 27 and 20 tons in 2008 and 2009. This decrease is mainly due to a decline in lead emissions through industrial processes. Lead emissions by traffic remained more or less the same during the last decade (2–3 tons/year) (VMM, 2012b). A similar declining trend in B-Pb levels is observed in other biomonitoring studies (Hrubá et al., 2012; Schulz et al., 2009) and is also visible in the consecutive reports of the CDC in the U.S. (CDC, 2009). A comparable trend is found for the B-Cd concentrations: lower concentrations are found in 2008–2009 (0.21 (0.19–0.23) µg/L), compared to the 2003–2004 campaign (0.36 (0.33–0.38 µg/L). Again, in the CDC reports, similar results are shown (CDC, 2009). However, as indicated above, conclusions about time trends should be made with the necessary caution since it is not excludable that differences in Cd and Pb concentrations between both campaigns are due to different sampling strategies.

When we compare B-Pb and B-Cd of Flemish adolescents with concentrations found in the international literature (in children of comparable age groups), both lead and cadmium concentrations are in the same range as the values found in the international literature, though lead concentrations are found at the lower end of the range. Concentrations of the other analysed trace metals in Flanders are in the same range as or lower than in other international studies.

Table 8
Comparison with FLEHS I and literature values.

µg/L	FL I	FL II	Ger[a]	Fr[b]	Sw[c]	Sw[d]	Sp[e]	US[f]	SA[g]
B-Cd	0.36	0.21	<0.12	0.31	0.28	0.11		<0.14	
U-Cd		0.24	0.08	0.16			0.49	0.12	
B-Pb	21.7	14.8	16.9	26	19	17.0		9.5	56.4
B-Cr		0.26				0.53			1.25
U-Cr							0.39		
B-Ni		1.3		2.1		2.8			
U-Ni		2.6	1.30	1.8			1.7		
B-As		0.62		5.0					1.53
U-As		12.3	4.3	19			1.36	8.6	
U-TRA		4.8		3.5[h]				3.7[i]	
B-Mn		9.7		7.6		12.0			8.48
B-Cu		790			950	900			1195
U-Cu		10.0		6.9			10.2		
B-Tl		0.027		0.02	<0.06	0.035			
U-Tl		0.20	0.26	0.15				0.20	
U-Sb		0.09	0.11	0.04				0.105	

FL = FLEHS.
[a] Germany: GerES 2003–2006, children aged 3–14, median (Schulz et al., 2009).
[b] France: Goullé et al. (2005), general population (median).
[c] Sweden: Bárány et al. (2001), children aged 15.
[d] Sweden: Rodushkin et al. (1999), aged 16–36.
[e] Spain: Aguilera et al. (2010), children aged 5–17.
[f] U.S.: NHANES 2003–2004 (CDC, 2009), children aged 12–19.
[g] South Africa: Bazzi et al. (2008).
[h] U.S.: Caldwell et al. (2009), aged 6–50 years.
[i] France: Fillol et al. (2010), >7 years.

Conclusions

Living in Genk-Zuid is associated with a significantly higher exposure to cadmium (18% higher in urine), chromium (32% higher in blood), copper (5% higher in blood, 11% higher in urine), thallium (11% higher in blood) and arsenic (toxic relevant arsenic 32% higher in urine). On the other hand, significantly lower concentrations are observed for antimony (21% lower in urine) and nickel (7% lower in urine). In Genk-Zuid, high concentrations of Cr, Ni, Mn and Cd are measured in fine dust particles. The negative correlation between the high atmospheric Ni values and the low Ni values in blood may be due to the low bioavailability of the atmospheric Ni compounds and the retention in the respiratory tract. Although Cu levels associated with PM_{10} are comparable to urban background levels in Flanders, B-Cu concentrations are highest in adolescents living closest to the industrial area, indicating that there is a Cu-emission source present. No explanation is found for the elevated B-Tl concentrations since there are no environmental data available. Although TRA is significantly elevated in urine, levels of arsenic associated with PM_{10} are low, and drinking water standards are nowhere exceeded. Also the comparison of dietary patterns cannot explain this result. The lower U-Sb concentrations observed in Genk-Zuid can be explained by the low levels measured in the environment.

Living in the area of Menen is associated with significantly higher exposure to thallium (27% higher in blood and 14% higher in urine), copper (6% higher in blood) and cadmium (28% higher in urine) but lower values were observed in Menen for lead (11% lower in blood), nickel (25% lower in urine), antimony (25% lower in urine) and arsenic (20% lower in urine). The metallurgical industry located in the North of France may be an important source of metals, transported towards Menen via the atmosphere and the Leie river. For Pb the situation in Menen is more complex: the lower Pb values in blood agree with the decreased atmospheric Pb concentrations, but not with those in the sediments and water column of the Leie river that are still high.

Compared to the previous biomonitoring programme FLEHS I (2002–2006) a decrease in the concentrations of Cd and especially of Pb in blood is found. This corresponds to the decreasing trend in atmospheric concentrations of these metals reported by VMM. However, it cannot be excluded that the differences between both campaigns are due to differences in sampling strategies. The average concentrations of manganese (in blood), copper (in blood and urine), thallium (in blood and urine), arsenic (in urine), toxic relevant arsenic (in urine) and antimony (in urine) are situated in the range of reported values in the international literature.

Acknowledgements

The studies of the Flemish Center of Expertise on Environment and Health are commissioned, financed and steered by the Ministry of the Flemish Community (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy).

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